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CELLULAR ANTIGENS IN THREE STOCKS OF *PEROMYSCUS MANICULATUS* FROM THE COLUMBIA RIVER VALLEY

BY PAUL A. MOODY

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INTRODUCTION

ONE stock of *Peromyscus maniculatus* from the north side of the Columbia River and two stocks from the south side of the river were investigated (1) to determine the occurrence and nature of intraspecific differences in the red blood cell antigens, and (2) to test the utility of the red cell agglutination reaction as an auxiliary to morphological investigations of the interrelationships of microgeographic races.

Antigenic differences between species within the genus *Peromyscus* have been demonstrated. Moody (1941) found such differences between the cells of *P. leucopus* and those of *P. maniculatus.* Cotterman (1944) employed normal human group A sera to differentiate a series of species of *Peromyscus* into two groups: one group, which included *maniculatus,* possessing an antigen similar to the human isoagglutinogen B, the other group lacking this antigen. Absorption of the sera with *P. polionotus* cells removed all antibodies capable of reacting with the latter but left agglutinins for cells of the closely related species *maniculatus* and of F_1 *maniculatus* × *polionotus* hybrids. Absorption of the sera with *maniculatus* cells removed agglutinins for cells of both species and of the hybrids. These results indicate antigenic differences between species of sufficiently close relationship so that ready hybridization occurs.

Similar tests of antigenic differences at the subspecific and infra-subspecific levels in *Peromyscus* appear not to have been made. Employing strains of laboratory mice (presumably varieties of *Mus musculus*) Gorer (1936*a*) distinguished with human group A sera two antigenic types and demonstrated that the difference depended upon a single gene. The same author (1936*b*; 1937) explored the antigenic constitution of three inbred strains of laboratory mice, employing immune sera much after the manner of the present investigation. He identified four antigenic factors variably distributed among the strains of mice investigated (*vide infra*).

ACKNOWLEDGMENTS

This study was conducted at the University of Vermont. I am grateful to Lee R. Dice and Harold J. Leraas for obtaining the ancestors of the stocks utilized. These mice were furnished by the Laboratory of Vertebrate Biology, University of Michigan. I am indebted to Dr. Dice for counsel in connection with the writing of the paper.

MATERIALS AND METHODS

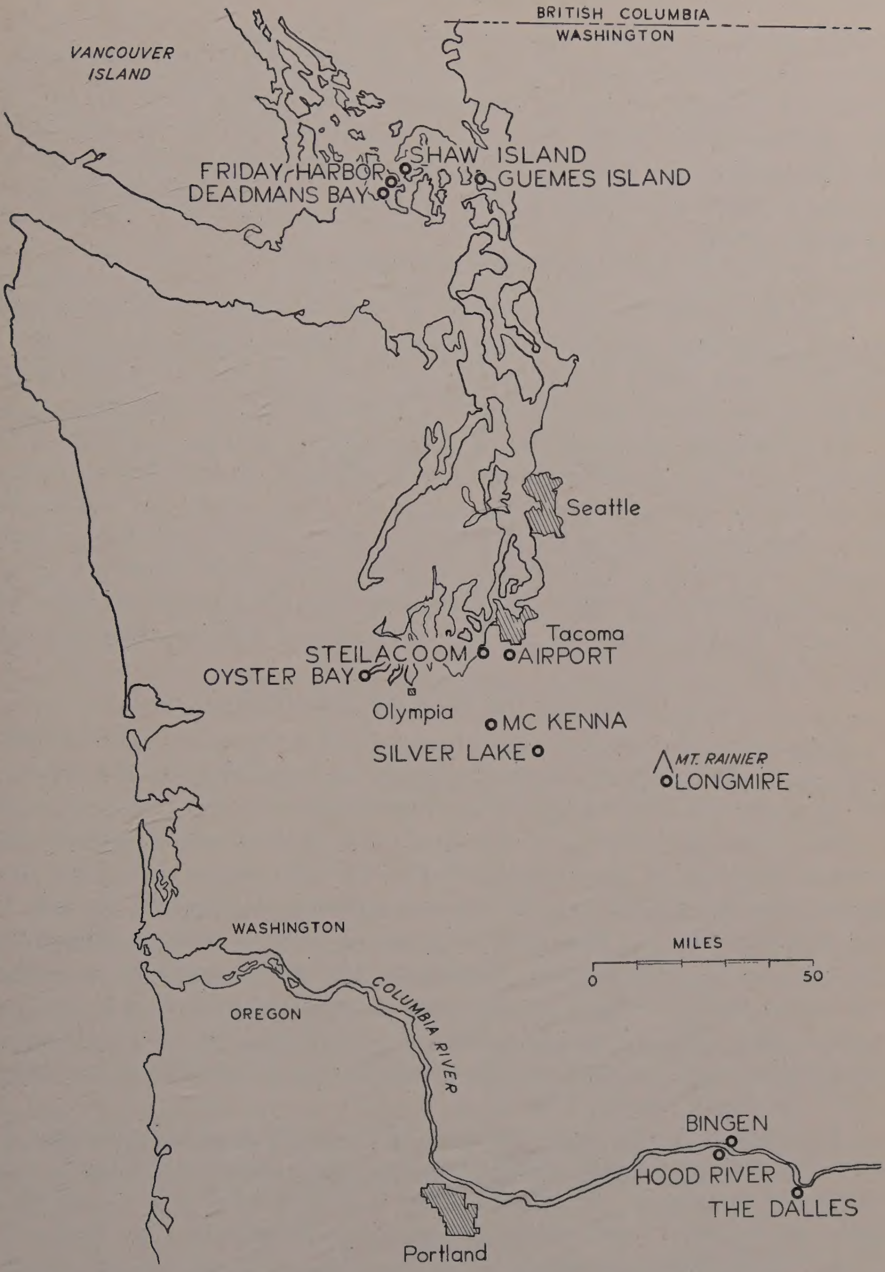
Stocks of Deer-Mice Included in the Study

The ancestors of three of the stocks employed were collected by Lee R. Dice during the summer of 1940. The stocks will be designated by the name of the locality from which each came. The three stocks from the Columbia River Valley originated, respectively, near the cities of The Dalles, Oregon, Hood River, Oregon, and Bingen, Washington. It will be noted from Map 1 that these localities are rather closely grouped geographically. Bingen and Hood River are almost directly across the river from each other; The Dalles is some twenty miles east of and on the same side of the Columbia River as is Hood River. Interest attaches to the question of whether or not the Columbia River here may form a barrier to the dispersal of deer-mice.

Dice (personal communication) has tentatively identified the Dalles and Bingen stocks as *P. maniculatus gambeli.* The Hood River stock is somewhat intermediate between *gambeli* and *rubidus*, but the larger number of characters points toward the latter subspecies rather than the former.

The Oyster Bay stock was collected in 1940 by Harold J. Leraas at a locality ten miles west of Olympia, Washington, on Oyster Bay. This stock is referred tentatively to *P. maniculatus austerus.*

Most of the mice utilized in the investigation were second and third generation descendants of the wild-caught animals. Although the stocks were not deliberately inbred, they were composed of the descendents of a relatively small number of individuals. Thus, it was not possible to be sure that each stock represented a truly random sample of the genotypes prevalent in the locality from which the stock was derived.



MAP 1. Western Washington and adjacent parts of Oregon, with localities from which the stocks of deer-mice originated.

Antiserum

The antiserum utilized was produced by inoculation of a rabbit with a 30 per cent suspension of pooled blood cells from mice of the Dalles stock. A total of 16.45 ml. of the cell suspension was inoculated intravenously in three six-day series. Blood was collected by cardiac puncture on the twelfth day following the last injection. The blood was defibrinated, and the serum was stored in sealed ampoules in the refrigerator. Technique was described by Moody (1941).

Absorption of Antiserum

Cells were collected from the mice by cardiac puncture, citrated, and washed three times with sterile 0.9 per cent buffered sodium chloride solution (cf. Moody, 1941). The blood from several mice was pooled, the number depending in part upon the quantity of antiserum to be absorbed.

The antiserum was diluted 1:100. Ten parts of diluted antiserum were usually added to one part of packed cells, although in final absorptions a proportion of 5:1 was frequently employed. Incubation was usually at room temperature for one or two hours, with frequent agitation. Occasionally, incubation at 37.5° C. for one hour was employed, though with no apparent difference in effect. At least two successive absorptions were required to remove completely antibodies capable of agglutinating a given type of cell.

Tests with the Absorbed Antiserum

Cells were collected as previously described and washed twice. They were used the day collected or the day following, being kept in the refrigerator meanwhile, to ensure against loss of sensitivity.

Tests were performed in 7 mm. test tubes using a modification of the method described by Boyd (1943: 360). Into each tube was pipetted 0.05 ml. of absorbed antiserum. To this was added a tiny loopful (a loop of No. 23 gauge Chromel wire made as small as possible without closing the opening) of the packed cells. Each tube was accompanied by a control containing 0.05 ml. of the physiological saline solution and a loopful of packed cells. The tubes were shaken, incubated for five minutes at room temperature with frequent additional shaking, and centrifuged for three minutes at approximately 2000 r.p.m.

The tests were read immediately. Macroscopic agglutination was observed by shaking the tubes gently until the cells in the control tubes became uniformly suspended. If cells in a test tube remained in a consolidated pellet the symbol "++++" was recorded; coarse flocculation was designated "+++," fine flocculation visible to the unaided eye, "++." Final readings were made with a binocular dissecting microscope employing 9 × oculars and 3.4 × objective. The stage was lighted from below with a substage microscope lamp. The tube to be read was placed on the stage in a holder which held the tube in a nearly horizontal position above a slit through which the light shone. Before being placed in the holder the tube was tilted so that the contents flowed toward the open end. The cells were viewed as they flowed back toward the closed end, the tube being in position in the holder above the lighted slit. Experience demonstrated that traces of agglutination could not be detected with certainty unless the cells were moving. If under the conditions described the agglutination had a granular appearance the symbol "+" was recorded. If the clumps were smaller and fewer the symbol "±" was used. In each case the tube containing antiserum was compared with the corresponding control tube. Traces of clotting sometimes simulated agglutination; error in this regard was avoided by observation of the control tube.

Apparently nature abhors an "endpoint." Reactions approach zero asymptotically. Agglutinations designated as "+" or "±" were definite and undoubted reactions, but between "±" and "–" a finely graded series of intermediate traces of agglutination was frequent. Despite my best efforts at objectivity, subjective errors in reading the tests undoubtedly occurred. In the interpretation of results faint traces of reaction were recorded as negative; only reactions of at least sufficient magnitude to be designated "±" were considered positive. Decision to adopt this criterion was influenced in part by the fact that at times faint traces of agglutination for the cells of a given individual remained even after such cells had been used twice in absorption of the antiserum. Apparently, absorption with pooled cells may not in every case remove all antibodies capable of reacting with the cells of every individual contributing to the pool. It seems, however, that this source of error, inherent in the use of pooled cells, was not significant in the present instance.

Absorbed Reagents

The diluted antiserum was first absorbed with cells of the Oyster Bay stock (*P. m. austerus*) to remove all antibodies for antigens shared in common by all members of the species *Peromyscus maniculatus*. Two successive absorptions, one incubated at room temperature the other at 37.5° C., were employed. In the first absorption macroscopic agglutination was immediate and heavy, seemingly involving the greater proportion of antibody content of the antiserum (Fig. 1).

Following absorption with cells of the Oyster Bay stock each reagent received subsequent additional absorption as described below.

RESULTS

Reagent I. This reagent was prepared by absorbing diluted antiserum with cells from the Oyster Bay stock, as described above, and then with cells from selected individuals of the Dalles stock. Cells of the individuals selected had been found in preliminary tests to be definitely but submaximally agglutinated by a sample of the antiserum absorbed with Oyster Bay cells and partly absorbed with Dalles cells.

Absorption of reagent I was continued until all antibodies capable of reacting with cells of all surviving Dalles individuals had been removed. Four successive absorptions were necessary. The reagent then contained antibodies capable of agglutinating cells of eight of twenty-nine Bingen mice tested, and of six of thirty-three Hood River mice tested (Table I). The antigenic component with which the residual antibodies in reagent I reacted is designated BH_2.

Reagent II. After preliminary absorption with cells of Oyster Bay mice, this sample of diluted antiserum was absorbed with Bingen cells which had been found in preliminary tests to be definitely but submaximally agglutinated by a sample of the antiserum absorbed with Oyster Bay cells and partly absorbed with Bingen cells. In later absorptions of reagent II cells which gave stronger reactions in the preliminary tests were used. Five successive absorptions removed all antibodies capable of reacting with cells of any surviving members of the Bingen stock.

The reagent then contained antibodies capable of agglutinating cells of ten of nineteen Dalles mice tested, and of five of nineteen Hood River mice tested (Table I). The antigenic component with which the residual antibodies in reagent II reacted is designated DH_2.

Reagent III. After preliminary absorption with Oyster Bay cells, this sample of antiserum was absorbed with Dalles cells found to contain DH_2 by tests with reagent II. Three absorptions removed all antibodies capable of agglutinating these cells.

The reagent was then found to contain antibodies capable of agglutinating cells of but two of the nineteen Dalles mice tested, and with the cells of these two the reaction was weak. The reagent agglutinated cells of twenty-one of twenty-five Bingen mice tested and of fourteen of seventeen Hood River mice tested (Table I). The antigenic component which reacted with reagent III is designated BH_1.

The tests indicate (Table I) that BH_1 is more widely distributed than is BH_2. BH_1 is present in many individuals which lack BH_2, but the converse never occurs, which seems to indicate some relationship between the two (see Discussion).

As far as the tests indicate, BH_2 is not present in any Dalles cells, whereas BH_1 is present in some of these cells. Evidence for this point is derived from two sources: by inference, since the Dalles cells used in absorbing reagent I removed BH_1, leaving BH_2; from the observation that the cells of two Dalles individuals were agglutinated, though weakly, by reagent III.

Reagent IV. After the preliminary absorption, this reagent was absorbed with Bingen cells found to contain BH_2 by tests with reagent I; all of them subsequently tested with reagent III contained BH_1 as well. Three

TABLE I

Results of Testing Cells of Individual Mice in Each of the Three Stocks, Using the Seven Reagents

See page 5 for explanation of symbols. Blanks in any column indicate that cells of the corresponding individuals were not tested with the reagent designed at the head of that column.

	Reagents						
	I	II	III	IV	V	VI	VII
Antigens	BH_2	DH_2	BH_1	DH_1	BD_1	DH_3	BD_2
Mice							
Dalles							
1144	−	±	−	+++	+	++	±
1146	−	−	±	++	+	±	−
1183	−	−	−	±			
1394	−	+	−	+			
1395	−	−	−	++	++		−
1396	−	−	−	±	±	+	
1397	−	−	−	+	+	+	+
1399	−	±	−				
1441	−	±	−	++	±	+	−
1442	−	+	−	++	+	−	−
1443	−	+	−	++	+	+	−
1485	−	−	±	+	++	+	−
1486	−	+	−	++			
1487	−	−	−	+	+	+	
1488	−	+	−	++	++	++	±
1489	−	−	−	−	++	−	−
1490	−	±	−	+++	+++	+	
1506							±
1508							+
1509							−
7033	−	−	−	±	++	±	
58164	−	+	−	+++	+	++	±
Bingen							
868	−	−	±	±	+	−	±
1134	−	−	+	++	+	−	−
1136	−	−	+	+			
1309	++	−	±	−	++	−	
1310	+						
1312	−	−	+	+	+	−	+
1314	+						
1316	+	−					
1318		−	±	−	+	−	−
1319	−	−	+	−	+	−	−
1320	−	−	−	+	+	−	
1321	+	−	±	−			
1322	−	−	+	+	−	−	
1325	−	−	+	+	−	−	
1326	++	−		−			
1363	++	−					
1365	−	−	+	+	+	−	
1366	−	−	+	+	+	−	+
1367	−	−	+	+	+	−	+
1368	−	−	+	+	+	−	+
1369	−	−	+	+	+	−	
Bingen							
1370	−	−	+	−			
1373	−	−	−	−	++	−	
1374	−	−	−	−	+	−	−
1470	−	−	±	+	+	−	
1471	++	−	±	−			
1472	−	−	++	+	+	−	−
1475	−	−	+	+	+	−	−
1476	−	−	−	+			
1477	−	−	+	+	±	−	±
Hood River							
1153	?						−
1154	?						−
1157	−	−	±	+	−	−	−
1158	−	−	±	+	−	−	−
1184	−	−	+	+	−	−	−
1185	−	±	+	++	−	−	
1186	−	−	±	+	−	−	
1187	−	−	±	+			
1272	−	−	+	++	−	−	
1273	−	−	−	+++	−	−	
1275	+	−	±	+	+	±	−
1276	?	−					−
1277	+	+	±	+++	+	+	
1278	?						−
1279	+	+	+	+	+	+	−
1280	−	−	+	±	−	−	−
1401	−						−
1402	−	−	±	+	−	+	
1403	?	−	−				−
1404	?						−
1405	+						−
1406	−	+	+	++	−	±	
1407	+	−	++	+	+	−	
1431	?						−
1432	?						−
1433	?	+	−				−
1435	−						−
1436	−						−
1437	?						−
1438	?						−
7619	−						−
7628	?						−
7898	±	−					

absorptions removed all antibodies capable of agglutinating the absorbing cells.

The reagent agglutinated cells of seventeen of twenty-six members of the Bingen stock tested, of all fifteen Hood River mice tested, and of seventeen of eighteen Dalles mice tested. This antigenic component is designated DH_1.

DH_1 is more widely distributed than is DH_2 (Table I); it is present in most members of the Bingen stock, all of which lack DH_2, and in a larger percentage of the members of the other two stocks than is DH_2. No individuals were found to possess DH_2 but not DH_1, although the converse occurred in many instances. This fact suggests a relationship between the two components which parallels the relationship between BH_1 and BH_2.

That the DH components are separate from the BH components is demonstrated by the facts (1) that no Dalles cells contained BH_2, whereas most of them contained DH_2, (2) that cells of but two Dalles mice tested contained BH_1 whereas cells of all but one of them contained DH_1, (3) that in tests of the other two stocks there seemed to be no regular relationship between the presence or absence of either of the DH components and the presence or absence of either of the BH components.

There is some evidence of cumulative action of DH_1 and DH_2. In many instances (Table I) macroscopic agglutination occurred with cells which contained both, although there were exceptions. Conversely, in most instances in which cells contained DH_1 but not DH_2 microscopic agglutination alone resulted; again, there were exceptions.

Reagent V. After preliminary absorption, this reagent was absorbed with Hood River cells which contained BH_1 and DH_1 but lacked BH_2 and DH_2. Two successive absorptions removed all antibodies capable of agglutinating these cells.

The reagent retained antibodies capable of agglutinating the cells of four of fourteen Hood River individuals tested, of all fifteen of the Dalles mice tested, and of eighteen of twenty Bingen mice tested (Table I). Apparently, the reaction was not due, primarily at least, to the presence of antibodies for BH_2 and DH_2. Cells of Hood River mice Nos. 1185 and 1406 were not agglutinated by reagent V, although tests with reagent II had shown that they contained DH_2. All Dalles cells had been found to lack BH_2, yet all were agglutinated by reagent V. All Bingen cells lacked DH_2 and most of them lacked BH_2, yet cells of all but two Bingen mice were agglutinated by reagent V. Finally, it is noteworthy that the cells of one Dalles individual (No. 1489) and of two Bingen individuals (Nos. 1373 and 1374) which contained none of the antigenic components previously identified were agglutinated, two of them strongly, by reagent V.

Evidently, therefore, reagent V contained antibodies against a component not previously isolated. This component is designated BD_1. Presumably, antibodies for BD_1 had been removed by all previous absorptions. This supposition is supported by the fact that the cells of all Dalles and Bingen individuals contributing to previous absorptions and extant for testing with reagent V were agglutinated by the latter.

Reagent VI. After preliminary absorption, this reagent was absorbed with Bingen cells which contained BH_1 and DH_1, but lacked BH_2 and DH_2. Three successive absorptions removed all antibodies capable of agglutinating these cells.

Reagent VI retained no antibodies capable of agglutinating cells of any of the Bingen mice tested. It agglutinated the cells of twelve of fourteen Dalles mice tested, and of five of fourteen Hood River mice tested (Table I). Reagent VI resembled reagent II in that all agglutinins for Bingen cells were removed; there seemed to be no correlation, however, between the reactions of the two reagents with cells of the other two stocks. Thus, the reaction produced by reagent VI was not dependent upon DH_2. Further evidence that agglutination could not have been due to DH_2 in some instances and BH_2 in others is afforded by the fact that the cells of one Hood River mouse and of six Dalles mice which contained neither component were agglutinated by reagent VI. Cells of Dalles No. 1442 and of Hood River No. 1185 possessed DH_2, but were not agglutinated by reagent VI. Similarly, cells of Bingen No. 1309 and of Hood River No. 1407 possessed BH_2, but were not agglutinated by reagent VI.

That the reaction of reagent VI could not have depended upon residual DH_1 was shown by the fact that cells of many of the Hood River mice which possessed DH_1 were not agglutinated by this reagent. Similarly, reaction did not depend upon residual BH_1, since many of the Dalles cells agglutinated by reagent VI lacked BH_1, and cells of several Hood River mice possessing BH_1 were not agglutinated by reagent VI. All but two of the individuals contributing cells for the absorption of reagent VI possessed component BD_1, hence antibodies for the latter must have been removed. Tests with reagent VI seemed to reveal no correlation with the results of tests with reagent V.

It seems evident, therefore, that the reaction of reagent VI depends upon an antigenic component not isolated in previous absorptions; this component is designated DH_3. As noted previously, no cells contained DH_2 which did not also contain DH_1. Similarly, no cells contained DH_3 which did not also contain DH_1, although many contained the latter without the former. These facts seem to indicate association between the three components which have been designated with "DH." Some cells contain DH_1 alone; some contain DH_1 and DH_2; some contain DH_1 and DH_3; some contain all three.

Reagent VII. After preliminary absorption, this reagent was absorbed with cells from the Hood River stock in an attempt to remove all antibodies capable of agglutinating cells of this stock. Three successive absorptions removed all agglutinins for cells of extant mice in the stock.

Reagent VII contained antibodies which agglutinated cells of six of fourteen Dalles mice tested, and of six of twelve Bingen mice tested (Table I). It was hoped that the absorption would remove antibodies for all antigenic components previously identified. Attainment of this goal was rendered difficult by the small number of animals remaining available, but one at least of the mice contributing to the absorption of this reagent (No. 1279) had been shown to possess all six of the components previously isolated. Although the tests are few and, consequently, not as conclusive as might be desired, it does not seem possible to ascribe the agglutination produced by reagent VII to any of the components identified previously; the antigenic component involved is therefore designated BD_2.

It will be noted that some cells contained BD_1 but not BD_2; the latter was never shown to occur without the former. Thus, it seems that there exists the same relationship between BD_1 and BD_2 as exists between BH_1 and BH_2, DH_1 and DH_2, or DH_1 and DH_3.

DISCUSSION

By use of parts of antiserum absorbed in seven different ways, seven different antigenic factors or components have been isolated. There is no evidence that the entire number present in erythrocytes of these stocks has been discovered or that the components which have been isolated have been resolved into their smallest constituent units.

The practical necessity of employing pooled cells for absorption renders difficult if not impossible the isolation of ultimate antigenic units dependent upon single genes after the manner of the human haemagglutinogens, the antigenic factors isolated in doves and pigeons by Irwin and coworkers (1936; 1939; 1940; 1943), or those identified in cattle by Ferguson (1941) and Ferguson, Stormont, and Irwin (1942). Gorer (1936*b*; 1937) mitigated the disadvantage imposed by use of pooled cells by employing stocks of mice which had been inbred, brother to sister, for over twenty generations. In consequence his stocks approached homozygosity, although the transplantation experiments of Loeb (1945: Chap. 9) suggest that prolonged inbreeding of mice does not result in as close an approach to identity of "individuality differential" as had sometimes been supposed. Nevertheless, the stocks employed by Gorer must have been much more uniform in antigenic structure than were the stocks used in the present investigation. Even approximate homozygosity can hardly be expected in any investigation upon mammals obtained from natural habitats or upon the immediate descendants of such animals.

Despite the handicaps imposed by the nature of the material, however, antigenic components in these stocks of deer-mice have been discovered, and some progress has been made toward analysis of their inter-relationships. The seven components occur in three groups or complexes. The "BH complex" (BH_1 and BH_2) comprises antigenic factors possessed in common by many Bingen and Hood River mice, but possessed by few, in the case of BH_1 or no, in the case of BH_2, Dalles mice. Similarly, the "DH complex" (DH_1, DH_2, DH_3) consists of components possessed in common by many Dalles and Hood River mice; DH_1 is also present in cells of a considerable proportion of Bingen mice, whereas DH_2 and DH_3 are absent from the latter. In like manner, the "BD complex" (BD_1 and BD_2) is composed of antigens shared by cells of many Bingen and Dalles mice; BD_1 is also present in cells of about one-fourth of the Hood River mice, and BD_2 is lacking in the latter stock.

A schematic representation of these complexes and components is given in Figure 1. The entire square represents the total antigen content of a red blood cell. The greater proportion of this content, represented by the lower two-thirds of the square, is shared in common by all mice of the species *Peromyscus maniculatus;* in this investigation the corresponding antibodies were removed by the preliminary absorption with the cells of the Oyster Bay stock. The three squares into which the upper third of the diagram is divided represent the three "complexes" described above; the subdivision of each into its constituent antigens is indicated. The cells of no one mouse contained all components included in the diagram.

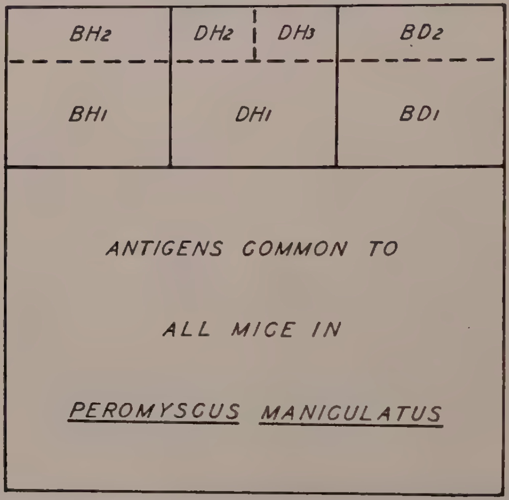


FIG. 1. Schematic representation of the relationships of the antigenic factors identified in the present study.

That these three "complexes" are real entities is suggested by a number of facts. In each one the antigens designated with the subscripts "2" and "3" were not found to occur in cells which did not have also the corresponding antigen designated with the subscript "1." The converse occurred frequently, however, as noted previously. The basic relationship of the component designated as "1" is also suggested by the results of the absorption of reagents V and VI. In each instance the attempt was made to remove agglutinins for BH_1 and DH_1, leaving antibodies for BH_2 and DH_2. The results seemed to indicate that antibodies for the latter were also removed, despite the fact that previous tests had failed to demonstrate the presence of BH_2 and DH_2 in cells of the kind used for absorption. Apparently, massive absorption with cells containing BH_1 (or DH_1) will remove not only antibodies for the latter but also antibodies for BH_2 (or DH_2, respectively). The amount of BH_1 in Dalles cells used to absorb reagent I was apparently too small to remove all antibodies for BH_2.

The nature of the relationship between the several components comprising one "complex" cannot be determined from the present investigation. Similarity of chemical structure would seem to offer the most reasonable explanation. There is much evidence that antibodies react not only with the antigen which elicited their formation but also with antigens of similar chemical structure (cf. Landsteiner, 1945: 54). According to this view DH_1 would be sufficiently similar to DH_2 so that antibodies for the latter could be removed by massive absorption with the former. Somewhat comparable situations are apparently presented by absorption of agglutinin α_1 from human sera of groups O or B by cells of group A which lack agglutinogen A_1 (Landsteiner and Witt, 1926), and by absorption of agglutinins for the factor N by human cells which are negative in the agglutination test for that factor (Landsteiner and Levine, 1928).

Of interest in this connection is the fact that although the antiserum was produced by inoculation of a rabbit with cells from Dalles mice, agglutinins were formed which were not completely absorbed by the cells of Dalles mice. As remarked by Boyd and Warshaver (1945): "It is certainly seldom that the observation is made that an antigen will not absorb out all the antibodies which it causes an animal to produce." Yet, in the present instance, the antiserum contained antibodies for a component (BH_2) seemingly not in the homologous (Dalles) cells, as well as for a component (BH_1) which was of apparently rare occurrence in them. Boyd and Warshaver (1945) reported four cases in which injection into rabbits of group B human erythrocytes resulted in the production of anti-A as well as of anti-B antibodies. They regarded the result as confirming the idea that the A and B antigens may be chemically similar. Their observations and mine accord well with the conception of Landsteiner (1945: 114 and 270–71), based upon experiments with immune sera for azoproteins and for ovalbumin (Landsteiner and van der Scheer, 1940), that the formation of several antibodies may be elicited in response to a single determinant group, that antibodies so formed "vary to some extent around a main pattern, and that what is ordinarily spoken of as an antibody is generally a mixture of specifically different components." Similar views have been expressed by other writers.

Since most of the mice which furnished cells for immunization of the rabbit did not survive for subsequent testing, there is some slight possibility that some of them possessed antigenic components not found in the Dalles mice which were tested. In view of the results of others this seems a less probable explanation for the occurrence of antibodies not completely absorbed by homologous cells than does the hypothesis given in the preceding paragraph.

Great individual variation occurred in the reaction of cells with the seven reagents (Table I). The cells of two individuals (Hood River Nos. 1277 and 1279) were agglutinated by six of the reagents. At the other extreme were individuals the cells of which were agglutinated by but one of the seven reagents. A variety of intermediate conditions existed. It is of interest that the two extremes were within a single stock (Hood River). This observation is in accord with the findings concerning agglutinogens in other animals (e.g., A and B in man) that differences between individuals within one race may be as great as differences between individuals belonging to different races.

Although the material of the present investigation does not lend itself to genetic analysis, some evidences of inheritance of antigenic components were found. Hood River mice Nos. 1277 and 1279, the only individuals that possessed six of the seven components, were sibs, another sibling being No. 1275, which possessed five components. At the other extreme was a litter mate, No. 1280, which possessed but two components. Nos. 1406 and 1407, offspring of the same parents as these others, possessed four components, variously assorted, the differences between the two individuals (Table I) suggesting the heterozygosity of the parents. Four of the five Hood River mice found to possess DH_3 were in this one family, as were all the Hood River mice that possessed BD_1. Somewhat similar family groupings occurred in the other stocks, but the present findings can serve merely to suggest interesting possibilities if future serological investigations on *Peromyscus* are planned to make genetic analysis possible.

Figure 2 presents "profiles" of the three stocks in terms of the percentages of individuals in each stock which possessed each of the seven agglutinogens. The percentages are of relative and approximate significance merely, since, as mentioned previously, there is no assurance that each stock presents a truly random sample of the population from which it was derived. Some essential points of difference between the stocks are emphasized by the "profiles." Each stock is characterized by possession in many individuals of two antigenic complexes, each shared largely with one of the other stocks and slightly, if at all, with the third one. Four of the components seem to be completely lacking from one or another of the stocks. Only one of the components (DH_1) has really considerable representation in all three stocks, although a second (BD_1) is present in a high percentage of the members of two stocks and in about 28 per cent of the third.

Thus, it is not possible to state that one stock is characterized by the presence in the cells of all individuals of an agglutinogen which is absent from the cells of all individuals of other stocks. A negative characterization is possible due to the fact that each stock may be said to be characterized by the absence of one or more agglutinogens present in the others. But on the whole the agglutinogens are shared by the stocks, each stock differing from the others in the proportion of the population possessing each of the antigenic factors. Characterization of stocks becomes statistical, therefore, as in the characterization of human racial groups by means of agglutinogens A and B.

In this respect the present findings agree with those of other similar investigations. Thus, Gorer (1936*b*; 1937), investigating inbred strains of agouti, albino, and black mice, isolated four antigenic factors. One of these was lacking from cells of the black strain; otherwise the four were present in all three strains, but the latter differed in the relative strength of the reaction by which the cells responded to antibodies for the respective factors. Landsteiner (1945: 117) investigated two species of ducks of such close relationship that hybrids were completely fertile. Using rabbit immune sera he was able "to demonstrate an agglutinable factor which occurs more frequently in one species but does not permit the differentiation of every individual." Owen, Stormont, and Irwin (1944) studied the differences in frequency between Guernsey and Holstein-Friesian cattle of thirty cellular

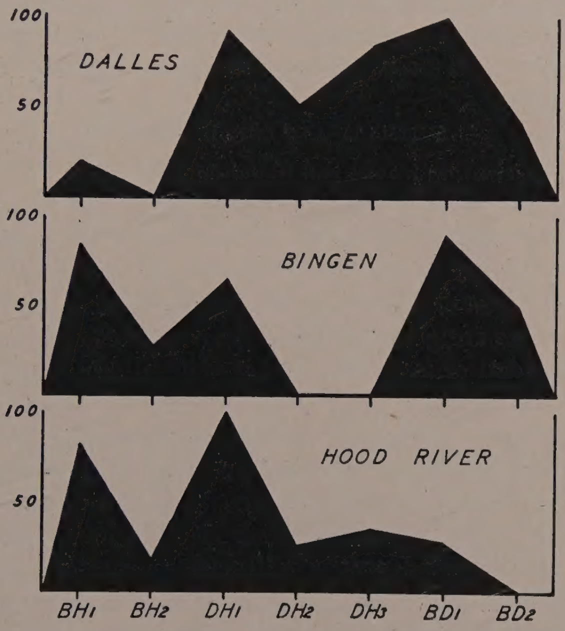


FIG. 2. Relative numbers of individuals in each of the three stocks that possessed each of the seven antigenic components. The ordinates are plotted in per cent of population exhibiting the components.

antigens. They reported that "no single antigen by itself distinguishes these two breeds. Rather, the breed differences in blood type are the result of quantitative differences in the frequencies of most of the antigens."

If the animals investigated are less closely related than were the ones used in the present investigation or those used in the studies cited immediately above, the overlapping in agglutinable substances is less marked and in consequence differentiation becomes more clear cut (cf. Landsteiner 1945: 78). Thus, Moody (1941) using absorbed antisera to identify individuals in two distinct species of *Peromyscus* found differentiation very definite. Apparently, most of the antigenic components of one species were not shared with the other. A few individuals gave evidence, however, of possessing a component shared with the other species, precisely in the manner in which the three closely related stocks of the present investigation possessed agglutinogens in common.

SUMMARY AND CONCLUSIONS

Antigenic constitution of the red blood cells of three stocks of deer-mice from the Columbia River Valley was investigated with absorbed reagents prepared from immune serum for the cells of one of the stocks. The three stocks were very similar in morphology, and this similarity extended to antigenic structure. Most of the cell antigens were shared by all the mice; when antibodies for these antigens had been removed from the antiserum, seven antigenic components of more limited distribution were identified (Table I and Fig. 1). Although four of these components were lacking from the cells of one or another of the stocks, for the most part the components were shared by the stocks, but possessed by varying proportions of the population of each.

Accordingly, characterization of the stocks must be mainly quantitative, rather than qualitative. The stocks resemble mosaics in which each of the constituent units varies independently in size, so that one pattern is never the duplicate of another. This mosaic pattern in distribution of antigens resembles the varying mosaics of morphological characters reported by Dice (1937: 17–20; 1939*a*; 1939*b*) to characterize local and geographical races. Thus, the average femur length of one local race may differ significantly from that of a neighboring local race. This difference may or may not be correlated with differences in other characters (e.g., length of mandible, length of ear, color of pelage). In like manner possession of an antigen in 90 per cent of the population may characterize one local race, possession of it in 10 per cent of the population may characterize another local race. This difference may or may not be correlated with differences in distribution of other antigens or with differences in morphological characters. Accordingly, the usefulness of cell antigens in studies of distribution and speciation will depend upon the nature of the problem at hand. The advantages and limitations are much the same as those which accompany attempts to apply to anthropological problems data concerning human blood groups and types.

Since all evidence points to genetic determination of antigens, it would seem that cell antigens might offer a valuable means by which to trace gene interchange between neighboring populations of mammals as well as the fluctuating distribution of genes throughout taxonomic and distributional groupings of higher order. A necessary preliminary to studies of this kind would be the isolation of antigenic units and determination of their genetic basis. From the results of investigations on other animals it would seem that isolation of antigens dependent upon single genes should be possible. For reasons discussed previously, pooling of blood from different individuals should be avoided if possible. If use of pooled cells proved a practical necessity, the raising of highly inbred strains as practiced by Gorer (1936*b*) would be essential. There would thus be made available requisite quantities of cells of uniform antigenic constitution for use in production of antisera and subsequent absorption of the latter. Sufficient quantities of uniform reagents for the testing of cells of wild populations could thus be secured.

The following observations are recorded and discussed: (1) individual differences in number of antigenic factors possessed were in some cases as great between members of the same stock as they were between members of different stocks; (2) under some circumstances absorption with cells containing one antigenic component removed antibodies for another one; (3) absorption with cells of the kind with which the rabbit producing the antiserum had been immunized did not remove all antibodies from the antiserum.

Two of the stocks (Dalles and Hood River) originated from the south side of the Columbia River; one (Bingen) came from the north side. A point of interest was whether or not the river in this region formed a barrier to the dispersal of deer-mice. The stocks were so similar in antigenic constitution that no evidence of separation by a barrier was afforded by the present investigation.

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University of Vermont, Burlington, Vermont.

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